HEPARIN-BINDING GROWTH FACTOR

Cross-Reference to Related Application

This is a continuation-in-part of application Ser. No. 07/462,156, filed January 8, 1990, now abandoned

Background of the Invention

This invention relates to a novel peptide growth factor and, more particularly, to a heparin-binding growth factor derived from bovine uterus and human placenta.

In recent years a considerable number of growth factors derived from various animal cells have been isolated and characterized. Illustrative of these growth factors are nerve growth factor (NGF) which has been purified from several different cell sources, insulin-like growth factors (IGF-I and IGF-11), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), endothelial cell growth factor (ECGF), somatomedins and transforming growth factors (TGF) derived from various tumors and virally transformed cells. For background information on these growth factors see, for example, the recent brief review articles by Kris et al., Biotechnology 3, 135-140 (1985); Dijk and Iwata, Ibid. 7, 793-798 (1989); and the comprehensive review in Hormonal Proteins and peptides, Ed. by Choh Hao Li, Vol. 12, "Growth Factors," Academic Press, 1984.

Although the existence of acidic and basic fibroblast growth factor (aFGF and bFGF) has been known for fifty years [Trowell, et al., J. Exp. Biol. 16, 60-70, (1939); Hoffman, Growth 4, 361-376

(1940)], only recently have they been purified and sequenced [Thomas et al., Proc. Natl. Acad. Sci. USA 81, 357-361 (1984); Gimenez-Gallego et al., Science 230, 1385-1388 (1985); Lemmon and Bradshaw, J. Cell. Biochem. 21, 195-208 (1983); Bohlen et al., Proc. Natl. Acad. Sci. USA 81, 5364-5368 (1984); and Esch et al., Proc. Natl. Acad. Sci. USA 82, 6507-6511 (1985)]. aFGF and bFGF have 55% sequence homology, suggesting that they arose by duplication and divergence from a common ancestral gene. Cells responding to the FGFs have between 10^3 and 10^5 high affinity receptors per cell [Neufeld and Gospodarowicz, J. Biol. Chem. 261, 5631-5637 (1986)]. The bFGF receptor has recently been purified, sequenced, and cloned [Lee et al., Science 245, 57-60 (1989)]. Both bFGF and aFGF compete for the same receptor and displace each other in radio-receptor assay [Neufeld, supra; Olwin and Hauschka, Biochemistry 25, 3487-3492 (1986)]; however, two forms of the receptor appear to have different affinities for aFGF and bFGF. Recently five additional proteins have been reported by cDNA cloning to have homology to the FGFs (hst, int-2, FGF-5, FGF-6, and KGF or FGF-7) [Dickson and Peters, Nature 326, 833 (1987); Yoshida et al., Proc. Natl. Acad. Sci. USA 84, 7305-7309 (1987); Delli Bovi et al., Cell 50, 729-737 (1987); Zhan et al., Mol. Cell. Biol. 8, 3487-3495 (1988); Marcis et al., Oncogene 4, 335-340 (1989); and Finch et al., Science 245, 752-755 (1989)]. All five cDNA sequences encode signal peptides, therefore suggesting that these five proteins are presumably secreted, unlike aFGF or bFGF which lack signal peptides.

Brief Description of the Invention

In accordance with the present invention a novel heparin-binding growth factor has been isolated from bovine uterus and human placenta. This novel heparin-binding growth factor, herein also designated as HBGF-8, is a 18.9 kDa polypeptide with a unique 25 N-terminal amino acid sequence as follows:

HBGF-8

Gly-Lys-Lys-Glu-Lys-Pro-Glu-Lys-Lys-Val-Lys-Lys-Ser-Asp-

Cys-Gly-Glu-Trp-Gln-Trp-Ser-Val-Cys-Val-Pro.

This novel growth factor binds tightly to cation exchange resins and to Heparin-Sepharose® and is stable to acetone precipitation and labile in acid.

HBGF-B was as active as acidic fibroblast growth factor (aFGF) and slightly less active than bFGF in the mouse NIH 3T3 fibroblast mitogenic assay system with an intrinsic specific activity of 5000 dpm/ng under standard assay conditions.

In an illustrative example and based upon total activity in the acetone extracts of bovine uterus stimulating ^{3}H-thymidine incorporation into DNA of serum-starved NIH 3T3 cells, a 6940 fold purification was achieved with an overall yield of HBFG-8 activity of 0.4%, using extraction of acetone powders and chromatographic separations at neutral pH. Approximately 18 µg protein was obtained from 1.2 kg wet weight of tissue. HBGF-8 was clearly separated from 17.5 kDa bovine uterus basic fibroblast growth factor (bFGF) by purification and its N-terminal amino acid sequence analysed. The unique sequence as set forth above was found.

In accordance with another aspect of the invention, the complete coding sequence of cDNA clones representing the full size bovine HBGF-8 and human HBGF-8 have been developed.

Thus, the bovine HBGF-8 cDNA sequence was isolated from bovine uterus cDNA library using the above bovine HBGF-8 N-terminal sequence clone as a probe. The cDNA sequence contains 1196 nucleotides and encodes a 18.9 kDa (M_r = 18,902) protein of 168 amino acids, including a 32 amino acid leader sequence. The cDNA coding sequence starts at nucleotide position 170 to 673.

The human HBGF-8 cDNA sequence was isolated from human placenta cDNA library using bovine HBGF-8 cDNA fragment as a probe. The cDNA sequence contains 995 nucleotides and encodes a 18.9 kDa (M_r = 18,942) protein of 168 amino acids, including a 32 amino acid lead sequence. The cDNA coding sequence starts at nucleotide position 252 to 755.

Comparison of the bovine and human HBGF-8 showed that HBGF-8 is highly conserved. Of 168 amino acids, 163 were identical. Differences occur at amino acid positions 3, 4 and 7 in the leader sequence and at amino acid positions 130 and 147 in the mature protein of the complete 168 amino acid sequence.

The human and bovine cDNA protein sequences and the human and bovine cDNA sequences for HBGF-8 are as follows:

Human HBGF-8 Protein

Met	Gln	Ala	Gln	Gln	Tyr	Gln	Gln	Gln	Arg	Arg	Lys	Phe	Ala	Ala	15
Ala	Phe	Leu	Ala	Phe	Ile	Phe	Ile	Leu	Ala	Ala	Val	Asp	Thr	Ala	30
Glu	Ala	Gly	Lys	Lys	Glu	Lys	Pro	Glu	Lys	Lys	Val	Lys	Lys	Ser	45
Asp	Cys	Gly	Glu	Trp	Gln	Trp	Ser	Val	Cys	Val	Pro	Thr	Ser	Gly	60
Asp	Cys	Gly	Leu	Gly	Thr	Arg	Glu	Gly	Thr	Arg	Thr	Gly	Ala	Glu	75
Cys	Lys	Gln	Thr	Met	Lys	Thr	Gln	Arg	Cys	Lys	Ile	Pro	Cys	Asn	90
Trp	Lys	Lys	Gln	Phe	Gly	Ala	Glu	Cys	Lys	Tyr	Gln	Phe	Gln	Ala	105
Trp	Gly	Glu	Cys	Asp	Leu	Asn	Thr	Ala	Leu	Lys	Thr	Arg	Thr	Gly	120
Ser	Leu	Lys	Arg	Ala	Leu	His	Asn	Ala	Glu	Cys	Gln	Lys	Thr	Val	135
Thr	Ile	Ser	Lys	Pro	Cys	Gly	Lys	Leu	Thr	Lys	Pro	Lys	Pro	Gln	150
Ala	Glu	Ser	Lys	Lys	Lys	Lys	Lys	Glu	Gly	Lys	Lys	Gln	Glu	Lys	165
Met	Leu	Asp													168

Bovine HBGF-8 Protein

Met	Gln	Thr	Pro	Gln	Tyr	Leu	Gln	Gln	Arg	Arg	Lys	Phe	Ala	Ala	15
Ala	Phe	Leu	Ala	Phe	Ile	Phe	Ile	Leu	Ala	Ala	Val	Asp	Thr	Ala	30
Glu	Ala	Gly	Lys	Lys	Glu	Lys	Pro	Glu	Lys	Lys	Val	Lys	Lys	Ser	45
Asp	Cys	Gly	Glu	Trp	Gln	Trp	Ser	Val	Cys	Val	Pro	Thr	Ser	Gly	60
Asp	Cys	Gly	Leu	Gly	Thr	Arg	Glu	Gly	Thr	Arg	Thr	Gly	Ala	Glu	75
Cys	Lys	Gln	Thr	Met	Lys	Thr	Gln	Arg	Cys	Lys	Ile	Pro	Cys	Asn	90
Trp	Lys	Lys	Gln	Phe	Gly	Ala	Glu	Cys	Lys	Tyr	Gln	Phe	Gln	Ala	105
Trp	Gly	Glu	Cys	Asp	Leu	Asn	Thr	Ala	Leu	Lys	Thr	Arg	Thr	Gly	120
Ser	Leu	Lys	Arg	Ala	Leu	His	Asn	Ala	Asp	Cys	Gln	Lys	Thr	Val	135
Thr	Ile	Ser	Lys	Pro	Cys	Gly	Lys	Leu	Thr	Lys	Ser	Lys	Pro	Gln	150
Ala	Glu	Ser	Lys	Lys	Lys	Lys	Lys	Glu	Gly	Lys	Lys	Gln	Glu	Lys	165
Met	Leu	Asp													168

HUMAN HBGF-8 cDNA

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GTCAAAGGCA GGATCAGGTT CCCCGCCTTC CAGTCCAAAA ATCCCGCCAA     50
GAGAGCCCCA GAGCAGAGGA AAATCCAAAG TGGAGAGAGG GGAAGAAAGA    100
GACCAGTGAG TCATCCGTCC AGAAGGCGGG GAGAGCAGCA GCGGCCCAAG    150
CAGGAGCTGC AGCGAGCCGG GTACCTGGAC TCAGCGGTAG CAACCTCGCC    200
CCTTGCAACA AAGGCAGACT GAGCGCCAGA GAGGACGTTT CCAACTCAAA    250
AATGCAGGCT CAACAGTACC AGCAGCAGCG TCGAAAATTT GCAGCTGCCT    300
TCTTGGCATT CATTTTCATA CTGGCAGCTG TGGATACTGC TGAAGCAGGG    350
AAGAAAGAGA AACCAGAAAA AAAAGTGAAG AAGTCTGACT GTGGAGAATG    400
GCAGTGGAGT GTGTGTGTGC CCACCAGTGG AGACTGTGGG CTGGGCACAC    450
GGGAGGGCAC TCGGACTGGA GCTGAGTCCA AGCAAACCAT GAAGACCCAG    500
AGATGTAAGA TCCCCTGCAA CTGGAAGAAG CAATTTGGCG CGGAGTGCAA    550
ATACCAGTTC CAGGCCTGGG GAGAATGTGA CCTGAACACA GCCCTGAAGA    600
CCAGAACTGG AAGTCTGAAG CGAGCCCTGC ACAATGCCGA ATGCCAGAAG    650
ACTGTCACCA CTCCCAAGCC CTGTGGCAAA CTGACCAAGC CCAAACCTCA    700
AGCAGAATCT AAGAAGAAGA AAAAGGAAGG CAAGAAACAG GAGAAGATGC    750
TGGATTAAAA GATGTCACCT GTGGAACATA AAAAGGACAT CAGCAAACAG    800
GATCAGTTAA CTATTGCATT TATATGTACC GTAGGCTTTG TATTCAAAAA    850
TTATCTATAG CTAAGTACAC AATAAGCAAA AACAAAAAGA AAAAAAAAAA    900
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    950
AAAAAAAAAA A                                              961
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Bovine HBGF-8 cDNA

GAGTGGAGAG AGTAGAAGAA AGAGAGCAGG GAGTCACCGG GCGTGCGGGG 50

GCGGAGAGCA GCGGCCGCCG CGAGCACCAG CGACTTGGGT ACCTGGACTC 100

AGGGCAGAAA AACCTCTCCC GGATCAACAA AGGCAGCCTG AGCGCGCACC 150

GCTCTTTTGC GACTCCAAAA TGCAGACTCC ACAGTACCTG CAGCAACGTC 200

GAAAATTTGC AGCTGCCTTT TTGGCATTTA TTTTCATCTT GGCAGCTGTG 250

GACACCGCTG AAGCAGGAAA GAAAGAGAAA CCAGAAAAGA AGGTGAAGAA 300

GTCTGACTGT GGAGAATGGC AGTGGAGTGT GTGTGTACCA ACCAGTGGGG 350

ACTGTGGGCT GGGCACCCGC GAGGGCACCC GTACCGGAGC TGAGTGTAAA 400

CAAACCATGA AGACCCAGAG ATGTAAGATC CCCTGCAACT GGAAAAAGCA 450

ATTTGGAGCG GAGTGCAAAT ACCAGTTCCA GGCCTGGGGA GAATGTGATC 500

TGAACACGGC TCTGAAGACC CGAACTGGGA GCCTGAAGCG AGCCCTCCAC 550

AACGCCGACT GCCAGAAGAC AGTCACCATC TCCAAGCCCT GTGGCAAGCT 600

GACCAAGTCC AAACCTCAAG CAGAATCTAA GAAGAAGAAA AAGGAAGGCA 650

AGAAACAGGA GAAGATGCTG GACTAAAAGC CACCACCTTC CGTGGACCAT 700

GAAAAGGACA TCAGCAAACA CGATCAGTTA ACTATTGCAT TTATATCTAC 750

CGTAGGCTTT TTATTCAAAA ATTATCTATA GCTTAAGTAC ACAATAGGCA 800

GAAACAAAAT GAAAAGAAAA ATTTTGTAGT AGCATTTTTT TTAAATGTAT 850

CAATATACCA TAGTACCACT AGGGACTTAT AATAGAGGAC TGTAATCCTA 900

TTTAGAATGT TGACTTATAG TACATGTTAA GTGATAGAAA ACTGAGGTAA 950

GTTTTTTGAA GTTATGTGAT ATTTTACATT ACATTTTTTT TTACATTTTT 1000

TTCTTTTGGC AGCAATTTAA ATGTTATGAC TATGTAAACT ACTTCTCTTG 1050

TTAGGTAATT TTTTTCACCT AGACTTTATT TCCCAATTGA GAAAAATATC 1100

TACTAAACAA AGCAGCAATA AAATATGATC ATCCTATCTG AGGAAAATAT 1150

CTCTTTTTCT GCCAGTGGAT TTTTAAAAAA TTGTAGTCAA CAAAAT 1196

In the bovine HBGF-8 protein sequence, the following amino acid replacements of the human ~~sequence~~ sequence are found:

Ala-3→Thr; Gln-4→Pro; Gln-7→Leu; Glu-130→Asp; and Pro-147→Ser.

Detailed Description of the Invention

While the specification concludes with claims particularly pointing out and distinctly claiming the subject matter regarded as forming the present invention, it is believed that the invention will be better understood from the following detailed description of preferred embodiments of the invention taken in connection with the accompanying drawings in which briefly:

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graphical representation which shows Chromatographic Separation of Bovine Uterus Mitogenic Activity. The mitogenic activity that bound to Heparin-Sepharose between 900 mM and 2000 mM NaCl was concentrated from 1160 ml to 12 ml using a MonoS HR 10/10 column, elution of this column using 2000 mM NaCl, and reapplication following dilution to 500 mM NaCl to the MonoS HR 5/5 column shown above. The A_{280} absorption profile elution of protein is shown by the shaded area. The results of the solid phase ELISA to detect bovine bFGF immunoreactivity are shown by the open triangles. In addition, the mitogenic activity in the mouse NIH 3T3 assay system is shown by the open squares. The NaCl gradient that was applied from 500 mM to 2000 mM salt is shown by the dashed line and individual fractions of 1 ml were collected. The bFGF immunoreacitivty is expressed as

µg/ml of bFGF. The region of the column shown by the continuous line and labeled region A was pooled, dialyzed, concentrated and applied to the microbore cation exchange column.

FIG. 2 is a graphical representation which shows cation exchange chromatographic separation of mitogenic proteins from bovine uterus. Region A (Fig. 1) was applied to a 2.1 x 220 nm Aquapore CX-300 cation exchanger and a 0-2 M NaCl gradient applied. The gradient is shown by the dashed line and the elution pattern of the proteins or A_{280} by the continuous line; the histogram shows the mitogenic activity of the fractions in the NIH 3T3 assay system. The HBGF-8 peak of mitogenic activity can be seen to elute at molarity of 1-1.2 M salt.

FIG. 3 shows the SDS-PAGE of the pure bovine uterus bFGF and HBGF-8. Lane 1 shows the appearance after SDS-PAGE in 15% gels of the pure 17.5 kD bovine uterus bFGF following reverse phase C4 chromatographic purification. This material was used for N-terminal sequencing. Lane 2 shows the appearance of the 17 kD novel HBGF-8 in the same gel system. This tube was used for determining novel N-terminal amino acid sequence shown above. The molecular weight markers used were BRL low molecular weight range markers.

In order to illustrate specific preferred embodiments of the invention in greater detail, the following exemplary laboratory preparative work was carried out. It will be appreciated, however, that the invention is not limited to these specific examples or the details described therein.

EXAMPLE 1

METHODS

Mitogenic Assays. The mitogenic activity of samples was assayed in triplicate by measuring the incorporation of [methyl-^{3}H] thymidine into acid-insoluble DNA of confluent, serum-starved NIH 3T3 cells. Unstimulated cells were used as background and values subtracted as background, which averaged 1500 dpm.

Immunological Methods. Rabbit anti-bFGF polyclonal antisera were obtained from Dr. Judith Abraham (California Biotechnology, Inc.). The solid phase immunosorbant assay used to screen column fractions was carried out by conventional procedure as previously described by Yeh et al., Proc. Natl. Acad. Sci. USA 84, 2317-2321 (1987).

Extraction of Growth Factor Activity from Acetone Powders. The acetone powders were prepared by conventional procedure as previously described by Porter et al., J. Biol. Chem. 263, 8989-8995 (1988), extracted with 40 ml/g of 0.9 M NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM [ethylenebis(oxyethylenenitrilo)] tetra-acetic acid (EGTA), 0.15 mg/ml phenylmethylsulphonyl fluoride. 10 mM benzamidine, 0.1 mg/ml soybean trypsin inhibitor, and 1 µg/ml aprotinin for 2 h at 4°C with agitation, and centrifuged at 10,000 x g for 30 min.

Heparin-Sepharose Chromatography. 6.9 liters of the soluble extract (34.5 g protein) was diluted to 20.7 liters in 900 mM NaCl, 20 mM Hepes, pH 7.0, loaded onto a 5 x 12.5 cm Heparin-Sepharose (Pharmacia) column at 4°C at a flow rate of 5 ml/min, washed in 10 column volumes of the starting buffer, 20 mM Hepes, pH 7.0, 900 mM NaCl, and eluted with 1160 ml of 20 mM Hepes, pH 7.0, 2 M NaCl, at a flow rate of 1 ml/min. The eluted fraction (2 M NaCl) was dialyzed against 20 mM MES, pH 6.0. Hepes = N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]. MES = 2[N-Morpholino]ethanesulfonic acid.

MonoS Chromatographic Separation of Mitogenic Activity. The active fraction (20 mM MES, pH 6.0) was loaded onto a MonoS HR 10/10 column (Pharmacia, FPLC) at 2.5 ml/min, washed with 10 column volumes of starting buffer, eluted with 12 ml (1.5 column volumes) of 20 mM MES, pH 6.0, 2 M NaCl, at a flow rate of 2.5 ml/min., diluted 1:4 with 20 mM MeS, pH 6.0 (final volume, 48 ml, 20 mM MES, pH 6.0, 500 mM NaCl), and loaded at 0.5 ml/min onto a MonoS HR 5/5 column (Pharmacia). Following loading, the column was washed with 10 column volumes of loading buffer and eluted with a 33 ml gradient from 500 mM NaCl to 2 M NaCl in 20 mM MES (pH 6.0). The column was further eluted with 2 M NaCl (10 ml) and the 1 ml fractions assayed in the NIH 3T3 mitogenic assay system. Each fraction also was assayed for bFGF immunoreactive proteins using a solid phase ELISA.

Microbore Cation Exchange Chromatography. The fractions (region A, Fig. 1, Mono HR 5/5 column) were pooled, dialyzed against 2 mM Hepes, pH 7.0, concentrated (1 ml), and loaded on a 2.1 x 220 mm aquapore CX-300 weak cation exchange column (Brownlee

Labs) at a flow rate of 200 µl/min (Applied Biosystems, Inc., Model 130A Microbore HPLC system). The column was eluted with a 0-2 M NaCl gradient (15 ml, 20 mM Hepes, pH 7.0) at a flow rate of 200 µl/min and fractions assayed in the NIH 3T3 assay system for mitogenic activity.

Reverse Phase Chromatographic Purification of bFGF. The fractions containing immunoreactive bFGF were loaded on a 2.1 x 220 nm Aquapore BU-300 C4 reverse phase column (Brownlee Labs, Applied Biosystems HPLC System), the column equilibrated with 100% water, 0.1% TFA, and eluted with a 15 ml gradient from 0-100% acetonitrile/0.1% TFA at a flow rate of 200 µl/min. TFA = trifluoroacetic acid.

N-Terminal Amino Acid Sequencing. Fractions were analyzed by repetitive sequence analysis on a gas phase sequencer (Model 470A, Applied Biosystems, Inc.) essentially as described by Hunkapiller et al., Meth. Enzymol. 91, 399-412 (1983). The respective PTH-aa derivatives were identified using an Applied Biosystems Model 120A PTH analyzer. The initial yields on sequencing both proteins were 185 and 2475 pmoles for the bFGF and HBGF-8, respectively. ~~Recoveries~~ Sequence purity averaged greater than 95% for both sequences.

RESULTS

Purification of Heparin-Binding Growth Factor-B (HBGF-8). The purification scheme for bovine uterine HBGF-8 is shown in Table 1. 1.2 kg of frozen bovine uterus yielded 18 µg of nearly pure HBGF-8 with an intrinsic specific activity in an NIH 3T3 cell mitogen assay of 5000 dpm/ng. Comparable values for aFGF and bFGF were 4090 and 9000 dpm/ng, respectively. Ten µg bFGF per 1.2 kg wet weight of bovine uterus was also obtained (see alternative method, above). An overall recovery of 0.4% of HBGF-B activity with a 6944 fold purification was obtained; because uterus contains other growth factors active in mitogenic assays using NIH 3T3 cells as targets, the recovery is grossly underestimated due to the initial inclusion of all growth factors active against NIH 3T3 cells in assay of crude extracts of bovine uterus.

The MonoS HR 5/5 column used to further separate the mitogenic proteins binding to Heparin-Sepharose is shown in Fig. 1. bFGF immunoreactivity and mitogenic activity are shown. bFGF eluted at 0.8 M NaCl; it is both immunoreactive and a potent mitogen in the NIH 3T3 assay system. Importantly, significant activity bound to MonoS more tightly than bFGF; this data first suggested the presence of a novel growth factor.

The fractions indicated by the solid bar, region A, were pooled, dialyzed, concentrated and loaded onto the microbore CX-300 cation exchange column for elution with a 0.0-2.0 M NaCl gradient.

The mitogenic activity identified (Fig. 2) corresponded to a 17 kD protein when analyzed in SDS-PAGE gels under reducing conditions. The appearance of the 17.5 kD bFGF and the novel 17 kD HBGF-8 by SDS-PAGE are shown in Fig. 3.

N-Terminal Amino Acid Sequencing. The separation of bFGF from HBGF-8 was pursued by N-terminal amino acid sequencing. The bFGF was further purified by C4 reverse phase chromatography to yield a single silver-stainable band at 17 kD on SDS-PAGE. N-terminal amino acid sequencing revealed N-terminal sequences for the bovine uterus bFGF proteins as shown in Table II. The N-terminal sequence confirmed that there is an N-terminal 2 amino acid extension beyond the first methionine sequenced (at position 3) which, during initial purifications of bFGF, was thought to be the site of initiation [Abraham et al., EMBO J. 5, 2523-2528 (1986)]. This N-terminal sequence obtained corresponded precisely with the N-terminal amino acid sequence for human placental bFGF [Sommer et al., Biochem. Biophys. Res. Commun. 144, 543-550 (1987)]. Alanine-2 in the bovine uterus bFGF sequence is substituted for threonine-2 in the human bFGF sequence.

A novel 25 amino acid N-terminal sequence was then obtained for HBGF-8, as shown in Table II, confirming the separation of two high affinity heparin-binding growth factors from bovine uterus. The sequence is extremely rich in lysines (7 of the first 12 amino acids). A search of both NBRF and GENBANK data bases failed to reveal sequence homology to any known protein or gene product. Thus, although

bFGF and HBGF-8 elute nearly together from various columns and migrate nearly identically by SDS-PAGE, each may be separated from the other and each have different N-terminal amino acid sequence.

HBGF-8 is both a potent mitogen and abundant in uterus. It has an intrinsic specific activity in the NIH 3T3 assay system which is roughly equal within methods of assay to aFGF and bFGF. aFGF could not be detected among the growth factors in bovine uterus which bound to Heparin-Sepharose at 900 mM NaCl. The 25 amino acid N-terminal sequence of HBGF-8 has not been identified in any of the seven members of the FGF family; aFGF [Gimenez-Gallego, supra.], bFGF [Sommer et al., supra.], int-2 [Dickson and Peters, supra.], hst [Yoshida et al., supra.], FGF-5 [Zhan et al., supra.], FGF-5 [Marcis et al., supra.], FGF-6 [Finch et al., supra.].

HBGF-8 was purified with aqueous columns with pH values between 6.0 and 7.0 About 18 µg/kg wet weight of tissue of HBGF-8 and ~ 10 µg/kg wet weight tissue of bFGF were recovered. However, it is likely that there may be more HBGF-8 in uterus than 18 µg/kg if all the activity which binds more tightly than bFGF in Fig. 1 is due to HBGF-8. Recently, aFGF has been shown to be more abundant than formerly thought with a tissue level of 500 µg/kg in heart [Sasaki et al., J. Biol. Chem. 264, 17606-17612 (1989)].

TABLE I. HBGF-8 purification scheme

	Total protein (mg)	Volume (ml)	Mitogenic activity* ($dpmx10^6$)	Specific activity§	Degree purification	Yield (%)
Solubilize acetone extract	†34,500	6,900	25,000	0.72	0	100
900-2000mM elution off heparin-sepharose	23	1,160	2,900	126	175	11.6
0-2000mM elution off MonoS HR 10/10	3	12	433	144	200	1.7
500-1000mM elution off MonoS HR 5/5	0.2	10	250	1250	1736	1.0
0-2000mM elution off CX-300 cation exchange column	0.018	0.6	90	5000	6944	0.4

*NIH 3T3 cell assay

§Units of specific activity are $dpmx10^6$/mg protein

†1.2Kg frozen bovine uterus yields 207g acetone insoluble powder, which on dissolving in 900M NaCl, 50mM Hepes pH 7.0, produced solution containing 34.5g of protein.

TABLE II. Bovine uterus bFGF and HBGF-8 N-terminal sequence

bFGF

80% GLY-ALA-MET-ALA-ALA-GLY-SER-ILE-THR-THR-LEU-PRO-ALA-LEU-
10% MET-ALA-ALA-GLY-SER-ILE-THR-THR-LEU-PRO-ALA-LEU-
10% PRO-ALA-LEU-

HBGF-8

95% GLY-LYS-LYS-GLU-LYS-PRO-GLU-LYS-LYS-VAL-LYS-LYS-SER-ASP-
CYS-GLY-GLU-TRP-GLN-TRP-SER-VAL-CYS-VAL-PRO.

EXAMPLE 2

Male term placenta was flash-frozen as 1 cm dice in liquid nitrogen and stored at -70°C. Total cellular and poly(A)+ RNA was prepared from placenta according to the conventional procedure of Chirgwin et al., Biochemistry 18, 5294-5299(1979), as modified by Sadler et al., Proc. Natl. Acad. Sci. USA 82, 6394-6398 (1985). cDNA was synthesized and constructed in λgt11 arms and packaged by the published method of Ye et al., J. Biol. Chem. 262, 3710-3725 (1987). Approximately 1 x 10^6 clones from the human placenta cDNA library were screened with a bovine cDNA probe. The screening procedure was as follows: the recombinant phages were replicated onto nitrocellulose filters and hybridized overnight at 42°C with random primer-labeled bovine cDNA probe using [α-^{32}P]dCTP (3,000 Ci/mmol, NEN), in 50% formamide, 50 mM sodium phosphate (pH 7.0), 5 x Denhardt's Solution, 0.1 mg/ml purified tRNA and 5x standard saline citrate (SSC) (1x SSC is 150 mM sodium

chloride, 15 mM sodium citrate). Filters were washed with 0.2x SSC and 0.1% SDS at 68°C and exposed to Kodak X-Omat AR film. After tertiary screening, a positive cDNA clone was subcloned into Bluescript (Stratagene). The nucleotide sequences were obtained by the well-known dideoxy chain termination method of Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463 (1977), with Sequenase version 2.0 (U.S. Biochemicals). The sequences were obtained by sequencing both strands of DNA twice with different primers.

The human HBGF-8 cDNA sequence revealed an open reading frame which encodes a putative 168 amino acid polypeptide with a calculated molecular weight of 18,942 daltons. A 25 amino acid sequence, which was identical to the experimentally determined N-terminal sequence of purified bovine HBGF-8, began 32 amino acids downstream of the proposed initiation codon. It also shows a hydrophobic N-terminal region of 32 amino acid residues which is believed to be a leader sequence. Comparison of predicted amino acid sequences of human and bovine HBGF-8 showed that HBGF-8 is highly converved. Of 168 amino acid residues, 163 are identical.

Standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyadenosine-5'-triphosphate (dATP). As is conventional for convenience in the structural representation of a DNA nucleotide sequence, only one strand is usually shown in which A on one strand connotes T on its complement and G connotes C.

Amino acids are shown herein by standard three letter abbreviations as follows:

Abbreviated Designation	Amino Acid
Ala	Alanine
Cys	Cysteine
Asp	Aspartic acid
Glu	Glutamic acid
Phe	Phenylalanine
Gly	Glycine
His	Histidine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine
Met	Methionine
Asn	Asparagine
Pro	Proline
Gln	Glutamine
Arg	Arginine
Ser	Serine
Thr	Threonine
Val	Valine
Trp	Tryptophan
Tyr	Tyrosine

Various other examples will be apparent to the person skilled in the art after reading the disclosure herein without departing from the spirit and scope of the invention and it is intended that all such examples be included within the scope of the appended claims.